SWORDTAIL MATE CHOICE AND REPRODUCTIVE ALLOCATION: EFFECTS OF MALE CONDITION

A Senior Scholars Thesis

by

SUZANNE ELYSE SIMPSON

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

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ABSTRACT

Swordtail Mate Choice and Reproductive Allocation: Effects of Male Condition.
(April 2011)

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We investigated the mechanisms behind reproductive allocation in sheepshead swordtail fish (*Xiphophorus birchmanni*). Classic life history theory predicts a trade-off between offspring size and number such that females with more offspring make smaller eggs and females with fewer eggs allocate more resources per egg. However, female swordtails with larger broods have been shown to designate more yolk per egg than those with fewer offspring. One explanation for this pattern is that females allocate differentially depending on the quality of their mate. We manipulated male diet to determine if females had a mate preference based on male chronic condition. Afterward, we dissected the females and measured their allocation using egg size and egg number. We found that females allocated more to fecundity when mated with males that were chronically underfed. This effect was strongest in females mated with low-food males that were exposed to high-food males. These results are unexpected and motivate future research on the relationship between mate quality and allocation to offspring size and number.

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I also want to thank Holly Kindsvater for helping me develop this research project and allowing me to use her field data on reproductive allocation. Additionally, Holly has also assisted with the statistical analysis and mate choice portions of the results and has been a constant supporter in all my research endeavors.

I want to thank the other undergraduates in the Rosenthal lab, especially Athena Mason and Courtney Passow, for their assistance with my project. Athena aided in my mate choice trials, while Courtney scrupulously fed my fish according to their prescribed diets.

Lastly, I want to thank my friends and family for supporting me through all the obstacles and joys this project has brought.

NOMENCLATURE

ANCOVA	Analysis of covariance
GLM	Generalized linear model
GLMM	Generalized linear mixed model
HF	High-food
LF	Low-food
R	Statistical program used for data analysis

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CHAPTER I

INTRODUCTION

The mechanisms behind reproductive allocation in sheepshead swordtail fish (*X. birchmanni*) are poorly understood, as they are counterintuitive to classical theory on resource conservation and allocation. Classical theory predicts a trade-off between offspring size and number such that females with more offspring make smaller eggs and females with fewer eggs allocate more resources per egg (Stearns 1992). However, female swordtails have contradicted this axiom without a clear explanation (Kindsvater, unpublished data).

Reproductive allocation is defined as the investment of resources for the purpose of reproduction (Allaby 2004). This investment is predicted to respond to environmental factors such as predation or the competition for food among fry (young fish). Each time a female reproduces, she must allocate resources based on offspring size and number. Ecological theory suggests that females would produce one size of offspring and vary number of offspring according to their resources. In contrast to this theory, large wild females have larger offspring than small females, and also produce more offspring (Kindsvater, unpublished data). Thus, additional factors besides predation and food competition must be driving the females' reproduction.

This thesis follows the style of *Functional Ecology*.

We hypothesized that females may be apportioning their resources according to the quality of their mates. In order to devise a system of understanding allocation in *X. birchmanni,* we set up an experiment that determined mate choice in females based on the quality of the males using the cues in male urine (Fisher & Rosenthal 2006b; Fisher & Rosenthal 2006a). After allowing them to mate, the fecundity of females was measured using egg size and number. If they displayed a mating preference and differed in fecundity, then a possible explanation for our question could be differential allocation. The differential allocation theory state that females distribute resources based on the quality of their mates and will invest great amounts of reproductive energy into what they perceive as a good mate (Sheldon 2000). This would explain why females do not incur a reproductive cost by producing a large number of heavily yolked eggs in a brood.

If the results indicate that larger females produce more eggs with greater masses, independent of mate quality, then a solution could be size-dependent allocation. This theory suggests that females with a larger standard length will have more reproductive resources than smaller females. However, this does not fully explain why females produce more expensive, larger offspring. If larger females produce larger broods in terms of egg size and number regardless of mate quality, females should undergo a reproductive energy cost and may not be able to mate as frequently. If smaller females give birth to fewer numbers of small offspring, and size is known to play a role in the reproductive success of *X. birchmanni* males*,* then the smaller females also incur a

fitness cost (Fisher, Mascuch & Rosenthal 2009). Thus, size-dependant allocation does not provide a complete explanation of the female's behavior.

To examine how male quality and female size influence female allocation to offspring size and number, we explored female allocation in a manipulative lab experiment using swordtails.

CHAPTER II

METHODS

Study population

In the experiment used for data analysis, approximately 35 males and 40 virgin females from the Rio Coacuilco (21.06'15"N, 98.35'12"W) population of *X. birchmanni* in Hidalgo, Mexico were used. All of the fish were lab-raised. In the initial study that did not collect significant data, there were 60 males and 64 females from the same population.

Initial diet preparation

The original goal of this study was to determine if females had a preference for males based on their recent versus chronic condition. To manipulate male condition, 60 males were placed on a "High-food" (HF) or "Low-food" (LF) diet with 30 HF and 30 LF. Within each group (HF or LF) the males were then divided into tanks with 5 males per tank. Thus we had 6 HF replicates and 6 LF replicates. The males were consistently fed their particular diet for at least a month. Each group of five HF males received 0.004g of brine shrimp in the morning and 0.2g of bloodworms in the afternoon. Each group of five LF males received 0.002g of brine shrimp and 0.1g of bloodworms. All replicates were fed at the same time.

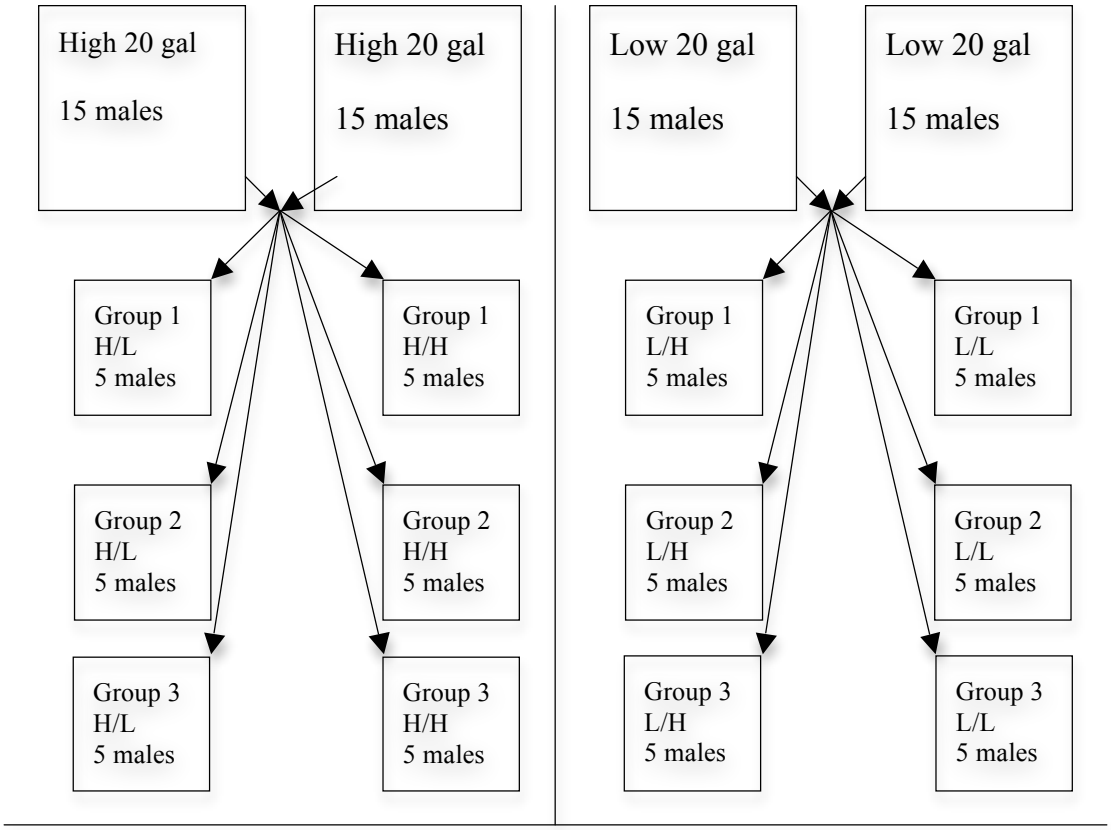

Figure 1: Flow-chart of diet switch.

After at least a month on their chronic diet, half of the HF males were switched to LF, while half continued HF feeding. Likewise, half of the LF males were switched to HF, while half continued their LF diet (Figure 1). The new diet continued for one week. The replicates (Group 1, 2, and 3) were switched sequentially over a period of three weeks to ensure there was enough time to complete the choice trials after a week of the new diet.

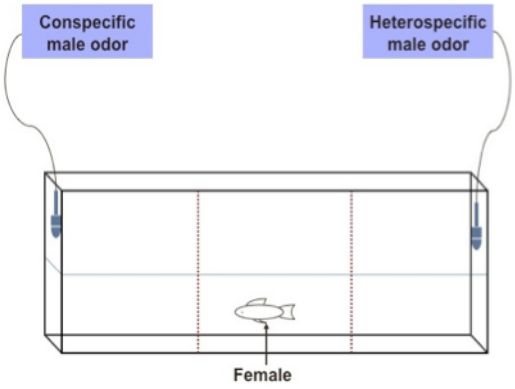

Fig. 2: Experimental setup for choice test trials. (Courtesy of Heidi Fisher)

Initial choice trials

After one week on the second diet, the male water served as cues for virgin female choice tests. Two tanks of size 76.2 x 20.3 x 20.3 cm were glued next to each other on the long side and filled about ¾ full with carbon-filtered water. Water temperature was between 67-73˚F. After a ten-minute acclimation period, a drip system released water from each experimental group of opposing sides of the tank. The cue the female had been exposed to was the conspecific cue and the unfamiliar cue was heterospecific (Figure 2). The female's behavior was recorded for ten minutes on primary and back-up webcams. The side on which the groups' water dripped was varied to account for side bias. After the trial the females were returned to their housing tanks. These choice tests had a low response rate and were not part of the data analysis. Females were exposed to males prior to the choice test in subsequent trials in order to stimulate their interest in mating.

Exposure treatment

The second type of choice trials performed used 35 males and 40 females. The males were divided in HF or LF diets with HF N=16 and LF N=19. They were fed the same portions as previously outlined. Males were split into groups of 3-5 fish and placed in 10-gallon tanks.

Each group of HF and LF males were exposed to, but did not directly interact with, a group of approximately five females. The females were fed brine shrimp and bloodworms normally. The females were housed in the same tanks as males but separated from them by porous Plexiglas. This allowed the fish to exchange water but prevented direct contact. This exposure treatment assured the sexual maturation of males and allowed females to become acquainted with the chemical cues of their male tankmates. It also ensured that both parties would have an interest in mating.

Exposure choice trials

At least one month after the males and females had been exposed to one another, the females were tested in choice trials. These followed the same procedure as the initial trials (Fig. 2). This data was used in the analysis.

Video analysis

The videos were analyzed to decipher a cue preference in females. The lanes were divided into a Right, Neutral, and Left zones by splitting the length of the tank into

thirds. The time a female spent in each area was timed with a stopwatch after the female had visited both sides of the tank (to account for side bias). A female that did not visit both sides of the tank at the end of the ten-minute period was considered unresponsive.

Fecundity analysis

After all the females had undergone choice tests, the divider between the males and females was removed and the fish were allowed to mate. Both males and females were fed normally from this point onward. After approximately two weeks of mating opportunity, the females were dissected and their eggs analyzed. The exposure period was two weeks to allow the fish enough time to mate as desired without neutralizing the chronic mate quality of the males. The females were then dissected and examined for the presence of eggs. The egg number, weight, and developmental stage were noted. Standard length was also measured from the tip of the snout to the end of the caudal peduncle.

Statistical analysis

The choice test data was analyzed using a nonparametric statistical test called the paired Wilcoxon signed rank test with the statistical program R. The first analysis used ANCOVA (analysis of covariance) to examine the effects of treatment in our exposure experiment. Female length was included as a covariate in this analysis.

The second analysis was done with a generalized linear mixed model (GLMM) to account for nesting effects (tanks) that cannot be done with ANCOVA. The GLMM also assumes that our data follows a Poisson distribution, meaning the data can never be negative. Our data fit this assumption because a female can never have a negative number of eggs.

CHAPTER III

RESULTS

Initial choice test results

In our first round of choice tests, we measured the response of virgin females to the cues of the experimental males. We were interested in whether females made reproductive decisions based on a male's recent condition (the diet that had occurred for a week) or chronic condition (the diet that had occurred for the previous month). We found, however, that the females were largely unresponsive (37.5% response rate) even when the drip rate and acclimation time were adjusted. We exposed the females to males in subsequent choice tests so they could form an interest in mating. It was also determined that the diet switch was too subtle of a cue to be accurately measured, leading to the exposure experiment. The data from the initial choice tests was not used in the analysis.

Exposure choice test results

The response rate for the exposure choice tests was 83%, vastly improve from the response rate in the initial experiment.

Mate choice

When analyzing the female preference for males in varying nutrient states, we found no significant effects of lane, side, or cue tank on the females' responses. Responsive females exposed to HF males (N=16) did not have a preference for either male cue

(Wilcoxon: p=0.782). Responsive females exposed to LF males (N=19) also did not have a preference for either cue (Wilcoxon: p=0.275). Additionally, when the data from each exposure group is pooled (N=35), no significant preference is discovered (p=0.50).

Fecundity

Since the full GLMM of effects of treatment on egg number estimate the tank effects (blocks) to be zero, we dropped them from subsequent analysis. Without random effects, the model is a generalized linear model, or GLM. Using a likelihood ratio test, we found a significant effect of female length when considered by itself and a significant interaction between length and female mate (Table 1). The effect of female exposure and its interactions lacked significance. The model prediction for the effect of female length and mate is seen in Figure 3. We also calculated the z-scores for female length (Wald test: $z_{39} = 2.081$; effect size = 0.094 +/- 0.045) and the interaction between female length and mate (Wald χ^2-test: $z_{39} = 2.027$; effect size = 0.15 +/- 0.073). Our results suggest a more significant correlation between female size and fecundity in matings with LF males.

Table 1: Fecundity Analysis of Deviance (Likelihood Ratio Test)

Model	**Deviance of Resid.**	**Df**	**Resid. Dev.**	**p (>|Chi|)**
Null		39	75.92	
Centered Length	*19.09*	*38*	*56.84*	*<0.0001*
Mate	1.8	37	55.66	0.28
Exposure	0.19	36	55.47	0.66
C length:Mate	*4.99*	*35*	*50.48*	*0.026*
C length:Exposure	0.014	34	50.46	0.91
Mate:Exposure	*3.11*	*33*	*47.35*	*0.078*
C length:Mate:Exposure	1.21	32	46.13	0.27

Italicized row indicates significant p-value (*p >0.05*)

Egg size

We undertook a similar strategy to analyze the effects of exposure and male chronic condition and egg dry mass. However, we assumed variance in egg size was normal and did not include female length. We again found the effects of treatment of offspring dry mass were predicted to be zero in the full GLMM and dropped them from subsequent analyses. Our final GLM included mate, female exposure, and the interaction between these two treatments. A likelihood ratio test determined the best model included mate, female exposure, and the interaction between them (Table 2). We found a significant effect of mate type and a significant interaction between mate and exposure. These results suggest an increase in embryo size in females who are mated to LF males with a stronger effect in females mated to LF after exposure to HF males.

Table 2: Embryo Dry Mass Analysis of Deviance (Likelihood Ratio Test)

Model	Deviance Resid.	Df	Resid. Deviance	p (>|F|)
Null		33	4.87e-05	
Exposure	2.50e-07	32	4.85e-05	0.66
Mate	6.2e-07	31	4.79e-05	0.49
Exposure:Mate	*9.1e-06*	*30*	*3.89e-05*	*0.013*

Italicized row indicates significant p-value ($p > 0.05$)

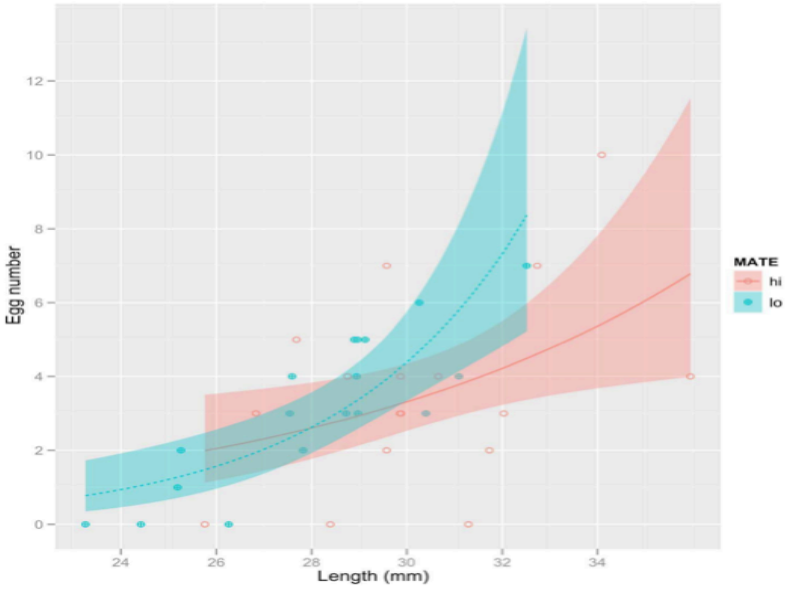

Fig. 3: Egg number vs. female length.

CHAPTER IV

CONCLUSIONS

Our results showed that females of greater standard length had larger broods with respect to number and weight. This means that these females exhibited the allocation pattern in question.

Choice test

The lack of preference in females for male cues differs from previous studies (Fisher & Rosenthal 2006a; Fisher & Rosenthal 2006b). This may be because our fish were not raised in social groups in the lab. It could also be evidence of a compounding variable within the study that caused the surprising results in fecundity. Further research is required to determine if females have a consistent preference for the cues of well-fed mates.

Fecundity

Our most surprising result was that females had higher fecundity when mated to LF males, particularly after exposure to HF males. This is opposite of what we would expect if females are basing their allocation on the quality of their mates.

Discussion

Females may have allocated more resources to lower-quality males to compensate for their mates (Gowaty 2008). Investing more resources into a lower quality mate could be a strategy females employ to reduce the fitness costs of reproducing with an inferior male. This would imply that the female only expects to mate once for that period of time.

To alleviate error in the choice tests, the male subjects should have developed secondary sexual characteristics (swords, body bars, dorsal fin height) or been raised in mixed-age groups to ensure they are mature and socialized. Since our fish were isolated, the males may not have been as fluent in courtship and mating protocols. This could have affected their production of cues in the water. However, regardless of the integrity of the cues, the females were indeed responding to some trait in the LF males that caused their increased allocation. Lower-quality mates perhaps displayed an increased aggression towards mating because of the urgency of their condition, and the females responded to a more enthusiastic mate. Research has indicated that hungry females have a stronger mating preference, and this characteristic could also extend to males (Fisher & Rosenthal 2006b). The stronger preference found in females exposed to HF males could be due to a partiality towards males that are dissimilar to their tankmates (an extension of the Coolidge effect) (Dewsbury 1981). This could be a technique to prevent inbreeding, however, it is not consistently seen throughout our study.

Further experiment is needed to support some of the conclusions drawn from our data. Repeating the choice tests with more mature fish that were raised in social groups may allow for data more similar to previous studies and allow for female fecundity to be more comprehensively analyzed. Using non-virgin females that have experience with mates may produce a reliable cue preference. The virgin females were used as a blank canvas to decipher if *X. birchmanni* has innate knowledge of reproductive decisions based on mate quality. While this possibility cannot be refuted, other options should be explored. If there continues to be a lack of evidence for a relationship between female allocation and mate quality, then the females may be responding to a different trait in the males when apportioning their reproductive resources.

The mechanisms behind female reproductive allocation in *X. birchmanni* are still puzzling. We have suggested several reasons for this behavior including compensation for a poor-quality mate, response to increased male aggression, and naïveté towards mating protocol due to inexperience or lack of proper socialization. Future studies should take these variables into account when continuing research.

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